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(57) Abstract

The present invention provides recombinant DNA containing a plant expressible gene which comprises in sequence: a promoter that drives expression of a downstream gene specifically in an initital feeding cell and/or a nematode feeding structure, a gene encoding a product that is inhibitory to an endogenous gene encoding a protein or polypeptide selected from the group consisting of ATP synthase, adenine nucleotide translocator, tricarboxylate translocator, dicarboxylate translocator, 2-oxo-glutarate translocator, cytochrome C, pyruvate kinase, glyceraldehyde-3P-dehydrogenase, NADPH-cytochrome p450 reductase, fatty acid synthase complex, glycerol-3P-acyltransferase, hydroxymethyl-glutaryl CoA reductase, aminocyl transferase, a transcription initiation factor, and a transcription elongation factor, a transcription terminator and a polyadenylation signal sequence, and wherein the said gene is expressed in said initial feeding cell or nematode feeding structure upon infection by the said nematode. The invention further provides plasmids, bacterial cells, recombinant plant genomes, as well as plant cells, plants and parts thereof, still harbouring recombinant genomes. The invention further provides plants with reduced susceptibilitiy against plant parasitic nematodes, as well as methods for obtaining same. Growing plants according to the invention in the field reduces yield losses due to nematode attack and/or reduces nematode populations in the soil.

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PLANTS WITH REDUCED SUSCEPTIBILITY TO PLANT-PARASITIC NEMATODES

TECHNICAL FIELD

This invention concerns plants with reduced susceptibility to plant-parasitic nematodes and methods for obtaining same involving recombinant DNA technology.

BACKGROUND OF THE INVENTION

Plant-parasitic nematodes worldwide cause diseases of nearly all crop plants of economic importance with estimated losses of about $ 5.8 billion/yr in the Unites States alone (Sasser and Freckman, 1987, World prospective on nematology In: Vistas on Nematology, Eds. Veech & Dickson. Hyatts Will, Maryland. pp. 7-14). While in tropical regions losses caused by nematodes are due mainly to root-knot nematodes (Meloidogyne), in Europe cyst nematodes of the genera Globodera and Heterodera are regarded as serious pests and important limiting factors in e.g. potato, rapeseed and sugarbeet cultivation, respectively. Only a small number of resistant crop varieties have emerged from breeding programmes for e.g. potato, sugarbeet, tomato, soybean and oil radish (Dropkin, 1988, Ann. Rev. Phytopath. 26, 145-161; Trudgill, 1991, Ann. Rev. Phytopath. 29, 167-192). The resistance is often based on single R-genes (Rick & Fobes, 1974, Tomato Gen. Coop. 24, 25; Barone et al. 1990, Mol. Gen. Genet. 224, 177-182) and leads to breakdown of resistance after several generations (Shidu & Webster, in: Plant Parasitic Nematodes, Vol. III, 1981, Zuckerman et al. (eds.) Acad. Press, New York, pp 61-87; Turner, 1990, Ann. Appl. Biol. 117, 385-397).

Plant-parasitic nematodes are obligate parasites. Nematodes such as cyst and root-knot nematodes are completely dependent on the formation of proper feeding structures inside the plant root. These feeding structures arise from single root cells that are selected by the nematode after invasion of the root. In the case of cyst nematodes, the IFC (initial feeding cell) develops into a syncytium through digestion of cell walls and hypertrophy. After infection with a root-knot nematode, the IFC develops into a giant cell through several nuclear divisions without cytokinesis and

becomes metabolically very active. During establishment of the feeding structure, the infective juvenile nematode becomes immobile and loses the ability to move to other feeding sites, illustrating their dependence on the induced nematode feeding structure (NFS).

Recent published methods to engineer nematode resistance (PCT WO92/04453) involve the modification of a gene that is specifically induced in the NFS after nematode infection. The local expression of phytotoxic genes would inhibit the development of the feeding structures, thus making a plant essentially resistant. This approach is strictly dependent on the availability of a promoter that is highly specific for nematode-induced feeding structures (NFS). Any promoter activity outside this structure will have adverse effects on plant development and crop yield. A highly NFS-specific promoter has been disclosed by Taylor et al. (1992, Proc. 31st Annual Meeting Amer. Soc. Nematologists, Vancouver Canada) and involves a truncated version of a root specific regulatory sequence (Δ0.3 TobRB7 as described in Yamamoto, 1991 Plant Cell 3; 371-382).

SUMMARY OF THE INVENTION

The invention provides recombinant DNA which comprises in sequence:

-a promoter that is capable of driving expression of a downstream gene specifically in an initial feeding cell and/or a nematode feeding structure,

-a gene encoding a product that is inhibitory to an endogenous gene encoding a protein or polypeptide selected from the group consisting of ATP synthase, adenine nucleotide translocator, tricarboxylate translocator, dicarboxylate translocator, 2-oxo-glutarate translocator, cytochrome C, pyruvate kinase, glyceraldehyde-3P-dehydrogenase, NADPH-cytochrome p450 reductase, fatty acid synthase complex, glycerol-3P-acyltransferase, hydroxymethyl-glutaryl CoA reductase, aminoacyl transferase, a transcription initiation factor, and a transcription elongation factor, and optionally

-a transcription terminator and a polyadenylation signal sequence,

and wherein the said gene is expressed in said initial feeding cell or nematode feeding structure upon infection by the said nematode. Preferred according to the invention is a recombinant DNA according to the invention, wherein said product comprises a RNA transcript that is complementary or partially complementary to the said endogenous gene transcript. A preferred nematode feeding structure-specific promoter according to the invention is one obtainable from the Delta-0.3TobRB7-5A promoter, joined to said inhibitory gene such that, upon infection of a plant parasitic nematode, the inhibitory gene is expressed specifically or predominantly in said nematode feeding structure.

The invention further provides a replicon comprising a recombinant DNA according to the invention, such as a Ti- or Ri-plasmid of an Agrobacterium species or a replicon capable of replication in E. coli and Agrobacterium species, a so-called binary vector system, as well as bacterium species, such as, Agrobacterium species, comprising a said replicon according to the invention.

Another embodiment of the invention is a plant genome which comprises a recombinant DNA according to the invention, as well as plant cells comprising same. Also preferred embodiments are plants comprising a cell or cells according to the invention. More preferred are plants regenerated from a cell according to the invention.

An especially preferred embodiment is a plant which, as a result of expression of a gene encoding a product that is inhibitory to an endogenous gene, shows reduced susceptibility to a plant parasitic nematode, preferably one which belongs to the family Solanaceae, more preferably the one is Solanum tuberosum, as well as plant material, such as flowers, fruit, leaves, pollen, seeds, or tubers, obtainable from a plant according to the invention.

The invention also provides a method for obtaining a plant with reduced susceptibility to a plant parasitic nematode, comprising the steps of

(1) transforming a recipient plant cell with recombinant DNA according to the invention,

(2) generating a plant from a transformed plant cell,

(3) identifying a transformed plant with reduced susceptibility to said plant parasitic nematode.

According to another aspect of the invention a method is provided for reducing damage to a crop due to plant parasitic nematodes, by growing plants according to the invention.

The meaning of the expressions used herein, as well as the application and the advantages of the invention will become clear from the following detailed description of the invention.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Binary vector pMOG23

Figure 2. Plasmid pMOG707, intermediate vector constructed for cloning purposes.

Figure 3. Restriction map of fragment from Ti-plasmid pTiB6

Figure 4. Intermediate vector pMOG579

Figure 5. Binary vectors pMOG711 - pMOG715. These plasmids are derivatives of pMOG23 and contain a truncated Δ0.3 TobRB7 promoter and an antisense construct of a gene that is essential for cell viability.

DETAILED DESCRIPTION

In the examples accompanying this description of the invention reduced susceptibility to plant parasitic nematodes is engineered in tobacco, potato and Arabidopsis plants by interfering with the autonomous, primary metabolism of cells comprising the feeding structure. In particular, the invention is outlined in somewhat more detail through antisense expression of the homologous gene coding for essential steps in primary metabolic pathways. For potato, the example is given for a gene coding for the mitochondrial adenine nucleotide translocator (Winning et al. 1992 Plant J. 2; 763-773). For Arabidopsis, the example is described for NADPH-cytochrome P450 reductase ATR2 (Mignote-Vieux et al. 1992 EMBL accession number X66017). For tobacco, the example is described using a gene coding for the beta subunit of the mitochondrial ATP-synthase (Boutry & Chua, 1985 EMBO J. 4; 2159-2165). The examples are described using the regulatory promoter sequence Δ0.3 (Taylor et al. 1992, Proc. 31st Ann.

Meeting Amer. Soc. Nematologists, Vancouver Canada) of the TobRB7 gene (Yamamoto et al. 1991, Plant Cell 3; 371-382) to ensure that expression is limited to the feeding structure.

Although exemplified in somewhat more detail for three plant species, the invention is not limited to any plant or nematode species.

Interference with autonomous primary metabolism brought about by disrupting genes inhibitory to an endogenous gene that encodes a protein or polypeptide product that is essential for cell viability. Disrupter genes according to the invention may be selected from such genes as formed by the group consisting of (a) genes encoding ribozymes against an endogenous RNA transcript, (b) genes which when transcribed produce RNA transcripts that are complementary or at least partially complementary to RNA transcripts of endogenous genes that are essential for cell viability, a method known as antisense inhibition of gene expression (disclosed in EP-A 240 208), or (c) genes that when transcribed produce RNA transcripts that are identical or at least very similar to transcripts of endogenous genes that are essential for cell viability, an as yet unknown way of inhibition of gene expression referred to as co-suppression (disclosed by Napoli C. et al., 1990, The Plant Cell 2, 279-289).

According to a preferred embodiment of the invention use is made of antisense genes to inhibit expression of endogenous genes essential for cell viability, which genes are expressed in the nematode feeding structures by virtue of a suitable nematode-specific promoter fused upstream to the said antisense gene.

Target genes for antisense disrupter genes are selected from those coding for enzymes that are essential for cell viability, also called housekeeping enzymes, and should be nuclear encoded, preferably as single copy genes, although a small size gene family would also be suitable for the purpose of the invention. Furthermore, the effect of antisense expression of said genes must not be nullified by diffusion or translocation from other cells or organelles of enzyme products normally synthesized by such enzymes. Preferably, genes coding for membrane-translocating enzymes are chosen as

these are involved in establishing chemical gradients across organellar membranes. Inhibition of such proteins by anti-sense expression can not, by definition, be cancelled by diffusion of substrates across the membrane in which these proteins reside. The translocated compound is not limited to organic molecules but can be of inorganic nature; e.g. P, H, OH or electrons.

Preferably, the membrane-translocating enzymes should be present in organelles that increase in numbers during parasitism, thereby illustrating the essential role that such organelles have in cells comprising the NFS. Specific examples for such organelles are mitochondria, endoplasmic reticulum and plasmodesmata (Hussey et al. 1992 Protoplasma 167;55-65, Magnusson & Golinowski 1991 Can. J. Botany 69;44-52). A list of target enzymes is given in Table 1 by way of example but the invention is not limited to the enzymes mentioned in this table. More detailed listings can be assembled from series as Biochemistry of Plants (Eds. Stumpf & Conn, 1988-1991, Vols. 1-16 Academic Press) or Encyclopedia of Plant Physiology (New Series, 1976, Springer-Verlag, Berlin).

Although only in some cases, the genes coding for these enzymes have been isolated and, therefore, the number of gene copies are not known, the criteria that have to be met are described in this invention.

TABLE 1
EXAMPLES OF TARGET ENZYMES FOR ANTISENSE EXPRESSION

enzyme	pathway/organelle
ATP synthase	mitochondrion
adenine nucleotide translocator	mitochondrion
phosphate translocator	mitochondrion
tricarboxylate translocator	mitochondrion
dicarboxylate translocator	mitochondrion
2-oxo-glutarate translocator	mitochondrion
cytochrome C	mitochondrion
pyruvate kinase	glycolysis
glyceraldehyde-3P-dehydrogenase	glycolysis
NADPH-cytochrome P450 reductase	lipid metabolism
fatty acid synthase complex	lipid metabolism
glycerol-3P-acyltransferase	lipid metabolism
hydroxymethyl-glutaryl CoA reductase	mevalonic acid pathway
aminoacyl transferase	nucleic acid metabolism
transcription factors	nucleic acid metabolism
elongation factors	nucleic acid metabolism

To maximize the antisense effects in a plant host, the use of homologous genes is preferred. With homologous is meant genes obtainable from the same plant species as the plant host. Heterologous, for the purpose of this specication shall mean obtainable from a different plant or non-plant species. Heterologous shall also comprise synthetic analogs of genes, modified in their mRNA encoding nucleic acid sequence to diverge at least 5% of the host gene. As house-keeping genes are in general highly conserved, heterologous probes from other (plant) species can be used to isolate the corresponding gene from the crop species that is to be made resistant. Such gene isolations are well within reach of those skilled in the art and, in view of the present teaching require no undue experimentation.

To differentiate between possible target genes and select favourable candidates to engineer nematode resistance, the following procedure can be applied by those skilled in the art: via the gene of interest, promoter-sequences can be isolated from genomic DNA and used for cloning in front of a

marker gene such as GUS (Jefferson et al. 1987 EMBO J. 6;3901-3907). This expression construct can then be and integrated into the plant genome. Regenerated plants can then be infected with PPN and used for histochemical GUS analysis of entire plants and the feeding structures in particular.

Alternative disrupter genes may be selected on the basis of the availability of mutants in unicellular eukaryotes such as yeast or Chlamydomonas can be used as indication. If for a particular enzyme, a large number of mutants are available then it is likely that this enzyme is redundant, present as multi-copy gene families, or that alternative pathways are available to circumvent the mutated enzyme (Strathern, Jones & Broach (Eds.) 1981 The molecular biology of the yeast Saccharomyces cerevisiae. Cold Spring Harbor Laboratory Press, New York). Such genes are less suitable for the methods described in this invention. By contrast, mutations in enzymes that are usually lethal for the recipient cell and therefor rarely available, indicate that an antisense deregulation of such genes will inhibit the proper development of that cell and can be used for the approach to engineer reduced susceptibility to PPN as disclosed in this invention. Gene disruption methods are available to test if a gene is essential for cell viability in which case the disruption event will be lethal (Rothstein, 1983 Methods Enzym. 101; 202-211). The homologous gene can then be isolated from the target crop with the yeast gene as a probe. Alternatively, the following promoter sequence can be used as nematode feeding site specific promoter; a truncated version of a tobacco root-specific promoter Δ0.3TobRB7 (Yamamoto et al. 1991 Plant Cell 3; 371-382). The full length sequence of the TobRB7 promoter is highly active inside NFS and this activity becomes more specific for the NFS when the truncated Δ0.3 version of the promoter is used (Taylor et al. 1992, Proc. 31st Ann. Meeting Amer. Soc. Nematologists, Vancouver Canada).

Other regulatory sequences such as terminator sequences and polyadenylation signals include any such sequence functioning as such in plants, the choice of which is within the level of skill of the average skilled person in the art. An

example of such sequences is the 3' flanking region of the nopaline synthase (nos) gene of Agrobacterium tumefaciens (Bevan, 1984, Nucl. Acids Res. 12, 8711-8721).A suitable promoter can be isolated via genes that are expressed at increased levels inside the NFS during nematode infection. Such genes can be isolated through differential screening of cDNA clones made from mRNA extracted from infected and healthy roots as was demonstrated for potato (Gurr S.J. et al. 1991, Mol. Gen. Genet. 226, 361-366). Although such promoters have never been described in detail, they can be selected and isolated in a well known manner from a plant by:

1. searching for a mRNA which is present primarily (although not necessarily exclusively) in infected root tissue,
2. isolating this mRNA
3. preparing a cDNA from this mRNA
4. using this cDNA as a probe to identify the regions in the plant genome which contain DNA coding for this specific mRNA
5. identifying and isolating the upstream (5') sequences from the DNA coding for this specific mRNA and that contains the promoter region.

Preferably, the infected roots used for mRNA isolation should be enriched for NFS e.g. by synchronous infection (Hammond-Kosack et al. 1989 Physiol. Mol. Plant. Pathol. 35, 495-506) or through direct isolation of feeding structures from plants in which NFS are visible at low magnification. For example feeding-structures that develop inside Arabidopsis roots can be seen at low magnification and are easy to isolate with a minimum of contaminating cells (Sijmons et al. 1991, Plant J. 1, 245-254). This allows the isolation, preferably using molecular enrichment procedures (Dickinson et al., 1991 Adv. Mol. Gen. Plant-Microbe Interact. 1 276-279) of genes corresponding to these RNA's and subsequent isolation of upstream promoter elements. Once identified, similar genes can be isolated from other plant species when the identified gene is used as a probe as in step 4. Species-specific upstream sequences can than be isolated from these other plant species

for use in a similar strategy as described in this invention. Upstream sequences of identified genomic clones can be fused to a gene for insertion in a suitable expression vector for plant transformation such as pMOG22 or pMOG23.

Alternatively, suitable promoters for expression of can be isolated via interposon tagging (Topping et al., 1991, Developm. 112, 1009-1019). In this approach, a number of different transgenic plants are regenerated after transformation with T-DNA from Agrobacterium carrying promoterless GUS constructs such as described by Topping et al. (1991, Developm. 112, 1009-1019) or pMOG452 as described in the Examples. After infection with a root-knot or cyst nematode and allowing some development of the NFS, roots can be stained for GUS activity. The random integration of the T-DNA enables the identification of promoter sequences that are active exclusively in the NFS. This type of interposon tagging of promoter sequences is especially well established in Arabidopsis (Kertbundit et al., 1991, Proc. Nat. Acad. Sci. USA 88, 5212-5216) and tobacco (Topping et al., 1991, Developm. 112, 1009-1019). The 5' upstream sequences responsible for GUS expression can be isolated with inverted polymerase chain reaction (inverted PCR) (Does et al. 1991, Plant Mol. Biol. 17, 151-153). Once suitable regulatory sequences are identified or genes that are transcribed inside NFS, they can be used as probes for the isolation of homologous sequences from other plant species. In turn, these sequences from other species can be fused to a disrupter gene for insertion in a suitable vector for plant transformation.

The application of this invention is not restricted to the plant species that are shown by way of demonstration. The choice of the plant species is primarily determined by the amount of damage through PPN infections estimated to occur in agriculture and the amenability of the plant species to transformation. Plant genera which are damaged during agricultural practice by PPN and which can be made significantly less susceptible to PPN by ways of the present invention include but are not limited to the genera mentioned in Table 2.

Nematode species as defined in the context of the

present invention belong to the superfamily Heteroderoidea and are divided among the families Heteroderidae and Meloidogynidae and include, but are not limited to the species mentioned in Table 2.

The choice of the plant species is primarily determined by the amount of damage through PPN infections estimated to occur in agriculture and the amenability of the plant species to transformation. Plant genera which are damaged during agricultural practice by PPN and which can be made significantly less susceptible to PPN by ways of the present invention include but are not limited to the genera mentioned in Table 2.

Nematode species as defined in the context of the present invention include all plant-parasitic nematodes that modify host cells into specially adapted feeding structures which range from migratory ectoparasites (e.g. Xiphinema spp.) to the more evolved sedentary endoparasites (e.g. Heteroderidae, Meloidogynae or Rotylenchulinae). A list of parasitic nematodes are given in Table 2, but the invention is not limited to the species mentioned in this table. More detailed listings are presented in Zuckerman et al. (eds., in: Plant Parasitic Nematodes, Vol. I 1971, New York, pp. 139-162).

The methods according to the invention to combat damage to crops due to nematode invasion is likewise applicable with non-nematode pests and pathogens, whenever said pathogen or pest locally down-regulates plant promoters at the site of infestation (e.g. in fungi-induced haustoria or aphid-induced galling). The principle of effecting the production of a neutralizing substance in all or most of the non-infestated plant parts to neutralize a cell disruptive substance the production of which is effected in at least the site of infestation, is independent of the type or species of the pathogen or pest.

TABLE 2
EXAMPLES OF PLANT-PARASITIC NEMATODES AND THEIR PRINCIPAL HOST PLANTS

Nematode Species	Principal Host Plants
Meloidogyne	
M. hapla	wide range
M. incognita	wide range
M. exigua	coffee, tea, Capsicum, Citrullus
M. indica	Citrus
M. javanica	wide range
M. africana	coffee
M. graminis	cereals, grasses
M. graminicola	rice
M. arenaria	wide range
Heterodera & Globodera	
H. mexicana	Lycopersicon esculentum, Solanum spp.
H. punctata	cereals, grasses
G. rostochiensis	Solanum tuberosum, Solanum spp, Lycopersicon esculentum
G. pallida	Solanum tuberosum
G. tabacum	Nicotiana tabacum, Nicotiana spp.
H. cajani	Cajanus cajan, Vigna sinensis
H. glycines	Glycine max, Glycine spp.
H. oryzae	Oryza sativa
H. schachtii	Beta spp, Brassica spp,
H. trifolii	Trifolium spp.
H. avenae	cereals, grasses
H. carotae	Daucus carota
H. cruciferae	Cruciferae
H. goettingiana	Pisum sativum, Vicia spp.

Within the context of this invention, a plant is said to show reduced susceptibility to PPN if a statistically significant decrease in the number of mature females developing at the surface of plant roots can be observed as compared to control plants. Susceptible / resistance classification according to the number of maturing females is standard practice both for cyst- and root-knöt nematodes (e.g. LaMondia, 1991, Plant Disease 75, 453-454; Omwega et al., 1990, Phytopathol. 80, 745-748).

The basic principle of reducing the plant's susceptibility to plant parasitic nematodes according to the invention is the manipulation of the nematode feeding structure. Manipulation of the nematode feeding structure for the purpose of this description of the invention shall include both preventing or retarding NFS formation as well as disrup-

tion once formation of the NFS is in an advanced stage. It is preferred to prevent or retard formation of the NFS, i.e. during the first stages of nematode invasion; to that end the NFS disruptive gene must be under the control of a promoter that drives expression at the onset of NFS formation.

However, in principle, it will also be acceptable if a disruptive gene is under the control of a promoter that drives expression of the disrupter gene in a more advanced stage of NFS formation causing the NFS to decline or to collapse. Either of these two extremes will provide the infected plant with decreased susceptibility towards the invading nematode. For the purpose of this invention the expression "disruption of the NFS" shall include retardation of NFS formation, decline of NFS formation once formed, or in the process of being formed, as well as total collapse of the NFS formed.

Reduced susceptibility to a plant parasitic nematode may be the result of a reduction of the number of NFS of the infected plant root, a reduction in the advancement of NFS formation, or a combination of both effects.

A nematode feeding structure according to the present invention shall include an initial feeding cell, which shall mean the cell or a very limited number of cells destined to become a nematode feeding structure, upon induction of the invading nematode.

A NFS disruptive effect according to the invention is not limited to adverse effects on the NFS only; also disruptive effects are contemplated that in addition have an adverse effect on nematode development by way of direct interaction.

Several techniques are available for the introduction of recombinant DNA containing the DNA sequences as described in the present invention into plant hosts. Such techniques include but are not limited to transformation of protoplasts using the calcium/polyethylene glycol method, electroporation and microinjection or (coated) particle bombardment (Potrykus, 1990, Bio/Technol. 8, 535-542).

In addition to these so-called direct DNA transformation

methods, transformation systems involving vectors are widely available, such as viral vectors (e.g. from the Cauliflower Mosaic Virus (CaMV) and bacterial vectors (e.g. from the genus Agrobacterium) (Potrykus, 1990, Bio/Technol. 8, 535-542). After selection and/or screening, the protoplasts, cells or plant parts that have been transformed can be regenerated into whole plants, using methods known in the art (Horsch et al., 1985, Science 225, 1229-1231). The choice of the transformation and/or regeneration techniques is not critical for this invention.

According to a preferred embodiment of the present invention use is made of so-called binary vector system (disclosed in EP-A 120 516) in which Agrobacterium strains are used which contain a helper plasmid with the virulence genes and a compatible plasmid, the binary vector, containing the gene construct to be transferred. This vector can replicate in both E.coli and in Agrobacterium; the one used here is derived from the binary vector Bin19 (Bevan, 1984, Nucl. Acids Res. 12, 8711-8721). The binary vectors as used in this example contain between the left- and right-border sequences of the T-DNA, an identical NPTII-gene coding for kanamycin resistance (Bevan, 1984, Nucl. Acids Res. 12, 8711-8721) and a multiple cloning site to clone in the required gene constructs.

The transformation and regeneration of monocotyledonous crops is not a standard procedure. However, recent scientific progress shows that in principle monocots are amenable to transformation and that fertile transgenic plants can be regenerated from transformed cells. The development of reproducible tissue culture systems for these crops, together with the powerful methods for introduction of genetic material into plant cells has facilitated transformation. Presently, preferred methods for transformation of monocots are microprojectile bombardment of explants or suspension cells, and direct DNA uptake or electroporation (Shimamoto, et al, 1989, Nature 338, 274-276). Transgenic maize plants have been obtained by introducing the Streptomyces hygroscopicus bar gene, which encodes phosphinothricin acetyltransferase (an enzyme which inactivates the herbicide phosphinothricin),

into embryogenic cells of a maize suspension culture by microparticle bombardment (Gordon-Kamm, 1990, Plant Cell, 2, 603-618). The introduction of genetic material into aleurone protoplasts of other monocot crops such as wheat and barley has been reported (Lee, 1989, Plant Mol. Biol. 13, 21-30). Wheat plants have been regenerated from embryogenic suspension culture by selection only the aged compact and nodular embryogenic callus tissues for the establishment of the embryogenic suspension cultures (Vasil, 1990 Bio/Technol. 8, 429-434). The combination with transformation systems for these crops enables the application of the present invention to monocots. These methods may also be applied for the transformation and regeneration of dicots.

Suitable selectable marker genes that can be used to select or screen for transformed cells, may be selected from any one of the following non-limitative list: neomycin phosphotranspherase genes conferring resistance to kanamycin (EP-B 131 623), the hygromycin resistance gene (EP 186 425 A2) the Glutathione-S-transferase gene from rat liver conferring resistance to glutathione derived herbicides (EP-A 256 223), glutamine synthetase conferring upon overexpression resistance to glutamine synthetase inhibitors such as phosphinothricin (WO87/05327), the acetyl transferase gene from Streptomyces viridochromogenes conferring resistance to the selective agent phosphinothricin (EP-A 275 957), the gene encoding a 5-enolshikimate-3-phosphate synthase (EPSPS) conferring tolerance to N-phosphonomethylglycine, the bar gene conferring resistance against Bialaphos (e.g. WO91/02071), and the like. The actual choice of the marker is not crucial as long as it is functional (i.e. selective) in combination with the plant cells of choice.

The marker gene and the gene of interest do not necessarily have to be linked, since co-transformation of unlinked genes (U.S. Patent 4,399,216) is also an efficient process in plant transformation.

The following examples are given only for purposes of illustration and do not intend to limit the scope of the invention. Unless otherwise stated in the Examples, all procedures for manipulating recombinant DNA were carried out

by using standard procedures as described in Sambrook et al. (Molecular Cloning, A laboratory Manual 2nd Edition, Cold Spring Harbor Laboratory (1990).

EXAMPLE I

Construction of cloning vectors

a) Construction of binary vector pMOG23

In this example the construction of the binary vector pMOG23 (in E. coli K-12 strain DH5, deposited at the Centraal Bureau voor Schimmel-cultures on January 29, 1990 under accession number CBS 102.90) is described.

The binary vector pMOG23 is a derivative of vector Bin19 (Bevan, 1984, Nucl. Acids Res. 12, 8711-8721). To obtain pMOG23, the vector Bin19 is changed in a way not essential for the present invention, using techniques familiar to those skilled in the art of molecular biology. First, the positions of the left border (LB) and the right border (RB) are switched with reference to the neomycine phosphotransferase gene II (NPTII gene). Secondly, the orientation of the NPTII gene is reversed giving transcription in the direction of LB. Finally the polylinker of Bin19 is replaced by a polylinker with the following restriction enzyme recognition sites: EcoRI, SmaI, BamHI, XbaI, SacI, XhoI and HindIII (Figure 1).

b) Construction of cloning vector pMOG707

A cloning vector pMOG707 is constructed, containing a right border T-DNA sequence, a multiple cloning site and a terminator for the purpose of cloning different promoter/gene combinations on a suitable fragment. This vector is constructed in the following manner: in the cloning vector pMTL26 (Chambers et al. 1988 Gene 68, 139-149) the XhoI site is removed by XhoI digestion, blunt-ended with Klenow polymerase followed by religation, resulting in pMTL26/2. This modified pMTL vector is used to clone the EcoRI - BglII fragment from pMOG23, containing the multiple cloning site and the right border sequences, resulting in pMOG584bis. The polylinker sequence is extended by inserting a synthetic linker between the BamHI and XhoI site, thus creating additional NcoI, XhoI and XbaI sites. Subsequently, the nopaline synthase trans-

cription terminator is isolated as a BamHI/HindIII fragment from the plasmid ROK1 (Baulcombe et al. 1986, Nature 321; 446), ligated to a synthetic adaptor such that the HindIII site is not recovered and an EcoRI site is introduced and subsequently cloned into the extended pMOG584bis as a BamHI - EcoRI fragment, resulting in plasmid pMOG707(Figure 2).

c) Mobilisation of binary vectors into Agrobacterium tumefaciens

The binary vectors described in Example IV-VIII are mobilized in a triparental mating with E. coli K-12 strain HB101 (containing plasmid RK2013) (Ditta et al., 1980, Proc. Nat. Acad. Sci. USA 77, 7347-7351), into Agrobacterium tumefaciens strains MOG101 (Example II) or LBA4404 (Hoekema et al. 1983, Nature 303, 179-180) that contains a plasmid with the virulence genes necessary for T-DNA transfer to plants.

EXAMPLE II

Construction of Agrobacterium strain MOG101

A binary vector system was used to transfer gene constructs into Arabidopsis plants. The helper plasmid conferring the Agrobacterium tumefaciens virulence functions was derived from the octopine Ti-plasmid pTiB6. MOG101 is a Agrobacterium tumefaciens strain carrying a non-oncogenic Ti-plasmid (Koekman et al. 1982, Plasmid 7, 119-132) from which the entire T-region was deleted and substituted by a bacterial Spectinomycin resistance marker from transposon Tn 1831 (Hooykaas et al., 1980 Plasmid 4, 64-75).

The Ti-plasmid pTiB6 contains two adjacent T-regions, TL (T-left) and TR (T-right). To obtain a derivative lacking the TL- and TR-regions, we constructed intermediate vector pMOG579. Plasmid pMOG579 is a pBR322 derivative, which contains the 2 Ti-plasmid fragments that are located to the left and right, outside the T-regions (Figure 3). The 2 fragments (shown in dark) are seperated in pMOG579 by a 2.5 kb BamHI - HindIII fragment from transposon Tn1831 (Hooykaas et al., 1980 Plasmid 4, 64-75) carrying the spectinomycin

resistance marker (Figure 4). The plasmid was introduced into Agrobacterium tumefaciens strain LBA1010 [C58-C9 (pTiB6) = a cured C58 strain in which pTiB6 was introduced (Koekman et al. 1982, Plasmid 7, 119-132), by triparental mating from E.coli, containing pRK2013 as a helper. Transconjugants were selected for resistance to Rifampicin (20 mg/l) and spectinomycin (250 mg/l). A double recombination between pMOG579 and pTiB6 resulted in loss of carbenicillin resistance (the pBR322 marker) and deletion of the entire T-region. Of 5000 spectinomycin resistant transconjugants replica plated onto carbenicillin (100 mg/l) 2 were found sensitive. Southern analysis showed that a double crossing over event had deleted the entire T-region (not shown). The resulting strain was called MOG101. This strain and its construction is analogous to strain GV2260 (Deblaere et al. 1985, Nucl. Acid Res. 13, 4777-4788).

EXAMPLE III

Isolation of a promoter fragment Delta0.3TobRB7 from tobacco

The Delta0.3TobRB7-5A promoter sequence (Yamamoto et al. 1991, Plant Cell 3: 371-382) was isolated by a two-step PCR on genomic DNA isolated from tobacco. In the first PCR reaction, part of the TobRB7-5A gene is being isolated using the following primers:

5' primer: 5' CTCCAAATACTAGCTCAAAACC 3' (SEQIDNO:1)

3' primer: 5' CCTCACCATGGTTAGTTCTC 3' (SEQIDNO:2).

The resulting PCR product is used to isolate the Delta0.3TobRB7-5A fragment using the following primers:

5' primer: 5' CTTGAATTCTAGATAAGCTTATCTAAAC 3' (SEQIDNO:3)

3' primer: 5' CCTCACCATGGTTAGTTCTC 3' (SEQIDNO:4).

The resulting PCR product is purified out of gel, blunt ended and subcloned into pUC9 (Vieira & Messing 1982 Gene 19; 259-268) which is then linearised with SmaI. Digestion of the resulting plasmid with XbaI and partially with NcoI yields the correct Delta0.3TobRB7-5A fragment for cloning in Examples IV-VIII.

EXAMPLE IV

Cloning of chimaeric DNA sequences of the Delta0.3 TobRB7 promoter and the antisense NADPH-Cytochrome P450 ATR1 gene for specific repression of the nematode-induced feeding structures in Arabidopsis.

a) cloning antisense NADPH-Cytochrome P450 ATR1 and construction of binary vector pMOG711

The clone for NADPH-cytochrome P450 reductase ATR1 (EMBL accession number X66016) is isolated from Arabidopsis thaliana var. Landsberg erecta using PCR technology on cDNA made of mRNA from this species. The primer set 5' GGCGGATCGGAGCGGGGAGCTGAAG 3' (SEQIDNO:5) and 5' GATACCATGGATCACCAGACATCTCTG 3' (SEQIDNO:6) is used to amplify the sequence of interest. This introduces a NcoI site on the N-terminus of the PCR fragment. Subsequently, the PCR fragment is digested with BamHI - NcoI and cloned antisense before the nopaline synthase terminator into pMOG707. The truncated promoter sequence Delta0.3TobRB7-5A (Yamamoto et al. 1991, Plant Cell 3; 371-382), isolated as described in Example IIIm, can then be inserted as a XbaI - NcoI fragment. The entire sequence is then cloned into the binary vector pMOG23 after digestion with EcoRI and one of the remaining unique restriction enzymes, resulting in binary vector pMOG711 (Figure 5).

b) expression of the Delta0.3 TobRB7/antisense NADPH-Cytochrome P450 ATR1 construct in Arabidopsis

Arabidopsis is transformed by cocultivation of plant tissue with Agrobacterium tumefaciens strain MOG101 containing the binary vector pMOG711. Transformation is carried out using cocultivation of Arabidopsis thaliana (ecotype C24) root segments as described by Valvekens et al. (1988, Proc. Nat. Acad. Sci. USA 85, 5536-5540). Transgenic plants are regenerated from shoots that grow on selection medium (50 mg/l kanamycin), rooted and transferred to germination medium or soil. Young plants can be grown to maturity and allowed to self-pollinate and set seed.

EXAMPLE V

Cloning of chimaeric DNA sequences of the Delta0.3 TobRB7 promoter and the antisense NADPH-Cytochrome P450 ATR2 gene for specific repression of the nematode-induced feeding structures in Arabidopsis.

a) cloning antisense NADPH-Cytochrome P450 ATR2 and construction of binary vector pMOG712

The clone for NADPH-cytochrome P450 reductase ATR2 (EMBL accession number X66017) is isolated from Arabidopsis thaliana var. Landsberg erecta using PCR technology on cDNA made of mRNA from this species. The primer set 5' GGTTCTGGGGATCCA-AAACGTGTCGAG 3' (SEQIDNO:7) and 5' GGCTTCCATGGTTTCGTTACCATACATC 3' (SEQIDNO:8) is used for amplification. This introduces both a BamHI and a NcoI flanking the PCR fragment. Subsequently, the PCR fragment is digested with BamHI - NcoI and cloned antisense before the nopaline synthase terminator into pMOG707. The truncated promoter sequence Delta0.3TobRB7-5A (Yamamoto et al. 1991, Plant Cell 3; 371-382), isolated as described in Example IIIm, can then be inserted as a XbaI - NcoI fragment. The entire sequence is then cloned into the binary vector pMOG23 after digestion with XhoI and partial digestion with EcoRI or, alternatively, after digestion with XbaI and partial digestion with EcoRI, resulting in binary vector pMOG712 (Figure 5).

b) expression of the Delta0.3 TobRB7/antisense NADPH-Cytochrome P450 ATR2 construct in Arabidopsis

Arabidopsis is transformed by cocultivation of plant tissue with Agrobacterium tumefaciens strain MOG101 containing the binary vector pMOG712. Transformation is carried out using cocultivation of Arabidopsis thaliana (ecotype C24) root segments as described by Valvekens et al. (1988, Proc. Nat. Acad. Sci. USA 85, 5536-5540). Transgenic plants are regenerated from shoots that grow on selection medium (50 mg/l kanamycin), rooted and transferred to germination medium or soil. Young plants can be grown to maturity and allowed to self-pollinate and set seed.

EXAMPLE VI

Cloning of chimaeric DNA sequences of the Delta0.3 TobRB7 promoter and the antisense glycerol-3-phosphate acyltransferase gene for specific repression of the nematode-induced feeding structures in Arabidopsis.

a) cloning antisense glycerol-3-phosphate acyltransferase and construction of binary vector pMOG713

The clone for glycerol-3-phosphate acyltransferase ATS1(EMBL accession number D00673) is isolated from Arabidopsis thaliana using PCR technology on cDNA made of mRNA from this species. The primer set 5' GCCCGGGATCCGGTTTATCCACTCG 3' (SEQIDNO:9) and 5' GAGTATTTTCCATGGATTGTGTTTGTG 3' (SEQIDNO:10) is used for amplification. This introduces both a SmaI, BamHI and a NcoI flanking the ATS1 clone. Subsequently, the PCR fragment is digested with SmaI - NcoI and as such subcloned into pMOG445. (pMOG445 is a pUC18 derivative that contains, by insertion of an oligo adaptor in the multiple cloning site, the extra restriction sites ClaI, NcoI and BglII between EcoRI and SstI). Subsequently, the ATS1 clone is isolated after NcoI and partial BamHI digestion and subcloned antisense before the nopaline synthase terminator into pMOG707. The truncated promoter sequence Delta0.3TobRB7-5A (Yamamoto et al. 1991, Plant Cell 3; 371-382), isolated as described in Example IIIm, is then inserted as a XbaI - NcoI fragment. The entire sequence is then cloned into the binary vector pMOG23 after digestion with EcoRI and one of the remaining unique restriction enzymes, resulting in binary vector pMOG713 (Figure 5).

b) expression of the Delta0.3 TobRB7/antisense NADPH-Cytochrome P450 ATR2 construct in Arabidopsis

Arabidopsis is transformed by cocultivation of plant tissue with Agrobacterium tumefaciens strain MOG101 containing the binary vector pMOG712. Transformation is carried out using cocultivation of Arabidopsis thaliana (ecotype C24) root segments as described by Valvekens et al. (1988, Proc. Nat. Acad. Sci. USA 85, 5536-5540). Transgenic plants are regenerated from shoots that grow on selection medium (50

mg/l kanamycin), rooted and transferred to germination medium or soil. Young plants can be grown to maturity and allowed to self-pollinate and set seed.

EXAMPLE VII

Cloning of chimaeric DNA sequences of the Delta0.3 TobRB7 promoter and the antisense adenine nucleotide translocator gene for specific repression of the nematode-induced feeding structures in potato.

a) cloning antisense adenine nucleotide translocator and construction of binary vector pMOG714

The clone for the mitochondrial adenine nucleotide translocator (PANT1, EMBL accession number X57557; Winning et al. 1992 Plant J. 2; 763-773) is isolated from Solanum tuberosum using PCR technology on cDNA made of mRNA from this species. The primer set 5' GCTAGCCGGATCCATCTGAGCTCCAG 3' (SEQIDNO:11) and 5' GACGTCCATGGCTGAATTAGCCACCACCG3' (SEQIDNO:12) is used for amplification. This introduces both a BamHI and a NcoI flanking the PANT1 clone. Subsequently, the PCR fragment is digested with BamHI - NcoI and cloned antisense before the nopaline synthase terminator into pMOG707. The truncated promoter sequence Delta0.3TobRB7-5A (Yamamoto et al. 1991, Plant Cell 3; 371-382), isolated as described in Example IIIm, is then be inserted as a XbaI - NcoI fragment. The entire sequence is then cloned into the binary vector pMOG23 after digestion with EcoRI and one of the remaining unique restriction sites, resulting in binary vector pMOG714 (Figure 5).

b) expression of the Delta0.3 TobRB7/antisense adenine nucleotide translocator construct in potato

Potato is transformed by cocultivation of plant tissue with Agrobacterium tumefaciens strain LBA4404 containing the binary vector pMOG714. Transformation is carried out using cocultivation of potato (Solanum tuberosum var. Desiree) tuber disks as described by Hoekema et al. 1989, Bio/Techn. 7, 273-278). Transgenic plants are regenerated from shoots that grow on selection medium (100 mg/l kanamycin), rooted,

multiplied axenically by meristem cuttings and transferred to soil to produce tubers.

EXAMPLE VIII

Cloning of chimaeric DNA sequences of the Delta0.3 TobRB7 promoter and the antisense ATP synthase gene for specific repression of the nematode-induced feeding structures in tobacco.

a) cloning antisense ATP synthase and construction of binary vector pMOG715

The clone for the beta subunit of ATP synthase (Boutry & Chua 1985 EMBO J. 4; 2159-2165) is isolated from tobacco (Nicotiana plumbaginifolia) using PCR technology on cDNA made of mRNA from this species. The primer set 5' CCCTCCAGGATCCCTTCTCGGAGGCTTC 3' (SEQIDNO:13) and 5' GAAAAGAAAGCCATGGAACTTTATAATC 3' (SEQIDNO:14) is used for amplification. This introduces both a BamHI and a NcoI flanking the ATP synthase clone. Subsequently, the PCR fragment is digested with BamHI - NcoI and cloned antisense before the nopaline synthase terminator into pMOG707. The truncated promoter sequence Delta0.3TobRB7-5A (Yamamoto et al. 1991, Plant Cell 3; 371-382), isolated as described in Example IIIm, is inserted as a XbaI - NcoI fragment. The entire sequence is then cloned into the binary vector pMOG23 after digestion with EcoRI and one of the remaining unique restriction sites, resulting in binary vector pMOG715 (Figure 5).

b) expression of the Delta0.3 TobRB7/antisense ATP synthase construct in tobacco

Tobacco is transformed by cocultivation of plant tissue with Agrobacterium tumefaciens strain LBA4404 (Hoekema et al. 1983, Nature 303, 179-180) containing the binary vector pMOG715 Transformation is carried out using cocultivation of tobacco (Nicotiana tabacum SR1) leaf disks as described by Horsch et al. 1985, Science 227, 1229-1231). Transgenic plants are regenerated from shoots that grow on selection medium (100 mg/l kanamycin), rooted and transferred to soil.

EXAMPLE IX

Analysis of transgenic Arabidopsis plants for susceptibility to SPPN

Transgenic Arabidopsis plants can be assayed both in vitro or in soil for resistance against M. incognita or the cyst nematode H. schachtii. For in vitro analysis, seeds are surface sterilized, grown and inoculated as described by Sijmons et al. (1991, Plant J. 1; 245-254). For soil-grown plants, seedlings are germinated on kanamycin-containing medium (10 mg/ml) and kanamycin-resistant seedlings are transferred to soil/sand mixtures (1:3 v/v) in 1x1x6 cm transparent plastic tubes. Once the rozettes are well developed (ca. 14 days) the containers are inoculated with ca. 300 hatched J2 of H. schachtii each. Eighteen days after inoculation, the roots are carefully removed from the soil/sand mixture and stained with acid fuchsin (Dropkin, 1989 in: Introduction to plant nematology, 2nd edition, Wiley & Sons, New York). In this assay, susceptible plants score a mean of 17 cysts per root system (range 4-40 cyst per root system). Similarly, plants can be inoculated with hatched J2 of M. incognita or with egg-masses that are mixed through the soil/sand mixture. The plants can than be scored for the presence of galls which are clearly visible once the roots are washed clear of the soil/sand mixture.

EXAMPLE X

Analysis of transgenic potato plants for susceptibility to SPPN

Transgenic potato plants can be assayed for resistance against M. incognita using soil that is preinfected with M. incognita egg masses mixed with sand (1:3 w/w), growing the potato plants in that soil mixture for 6 weeks and , after removing the soil, count the developed number of galls on a root system. Alternatively, to assay for resistance against Globodera ssp. a closed container is used. For this assay, three replicate 2-4 cm tubers are transferred to soil which is pre-inoculated with cysts from G. rostochiensis or G. pallida in transparent containers. The peripheral root systems can be analyzed visually 7-8 weeks after germination

for the presence of cysts. A genotype will be scored as resistant if none of the three replicates had cysts and susceptible if at least one of the three replicates shows cysts.

EXAMPLE XI

Analysis of transgenic tobacco plants for susceptibility to SPPN

For anlysis of nematode resistance, the soil is preinfected with M. incognita egg masses. This inoculum can be produced by maintaining a stock culture of M. incognita on soil grown celery plants (Apium graveolens) under standard greenhouse conditions, below 25°C. Mature celery root systems, containing a high number of root knots and mature females of M. incognita, are carefully dusted off to remove the soil, homogenized briefly in a Waring blendor (2 seconds) and weighed in portions of 60 gram. These root samples are mixed with 1 kg sand:potting soil (1:1) mixtures and used for growth of transgenic tobacco transformants. As control plants, primary kanamycin resistant transformants (transgenic for pMOG23) are used. Per construct, 100 primary transformants are grown in infected soil for 6 weeks. The soil/sand mixture is washed away carefully and the number of galls / root system is counted with a binocular. Control plants have a mean of 25 ± 11 galls. A genotype is considered resistant when the mean number of galls is reduced to 2 per root system. The primary transformants meet this requirement, can than be used for a rapid multiplication cycle by placing transformed leaves again on media that allows shoot regeneration (Horsch et al. 1985, Science 227, 1229-1231) or the plants can be grown to maturity and allowed to flower and seed setting and used for more extensive testing of nematode resistance using 100 plants of each genotype.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: MOGEN International N.V.
(B) STREET: Einsteinweg 97
(C) CITY: LEIDEN
(D) STATE: Zuid-Holland
(E) COUNTRY: The Netherlands
(F) POSTAL CODE (ZIP): NL-2333 CB
(G) TELEPHONE: (0)31.71.258282
(H) TELEFAX: (0)31.71.221471
(I) TELEX: -

(ii) TITLE OF INVENTION: PLANTS WITH REDUCED SUSCEPTIBILITY TO PLANT-PARASITIC NEMATODES

(iii) NUMBER OF SEQUENCES: 14

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(v) CURRENT APPLICATION DATA:
APPLICATION NUMBER: EP 92203378.2

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

CTCCAAATAC TAGCTCAAAA CC 22

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

CCTCACCATG GTTAGTTCTC 20

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CTTGAATTCT AGATAAGCTT ATCTAAAC 28

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CCTCACCATG GTTAGTTCTC 20

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GGCGGATCGG AGCGGGGAGC TGAAG 25

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GATACCATGG ATCACCAGAC ATCTCTG 27

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GGTTCTGGGG ATCCAAAACG TGTCGAG 27

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GGCTTCCATG GTTTCGTTAC CATACATC 28

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GCCCGGGATC CGGTTTATCC ACTCG 25

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GAGTATTTTC CATGGATTGT GTTTGTG 27

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GCTAGCCGGA TCCATCTGAG CTCCAG 26

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GACGTCCATG GCTGAATTAG CCACCACCG 29

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

CCCTCCAGGA TCCCTTCTCG GAGGCTTC 28

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GAAAAGAAAG CCATGGAACT TTATAATC 28

CLAIMS

1. A recombinant DNA containing a plant expressible gene which comprises in sequence:
-a promoter that is capable of driving expression of a downstream gene specifically in an initial feeding cell and/or a nematode feeding structure,
-a gene encoding a product that is inhibitory to an endogenous gene encoding a protein or polypeptide selected from the group consisting of ATP synthase, adenine nucleotide translocator, tricarboxylate translocator, dicarboxylate translocator, 2-oxo-glutarate translocator, cytochrome C, pyruvate kinase, glyceraldehyde-3P-dehydrogenase, NADPH-cytochrome p450 reductase, fatty acid synthase complex, glycerol-3P-acyltransferase, hydroxymethyl-glutaryl CoA reductase, aminoacyl transferase, a transcription initiation factor, and a transcription elongation factor, and optionally
-a transcription terminator and a polyadenylation signal sequence,
and wherein the said gene is expressed in said initial feeding cell or nematode feeding structure upon infection by the said nematode.

2. A recombinant DNA according to claim 2, wherein said product comprises a RNA transcript that is complementary or partially complementary to the said endogenous gene transcript.

3. A recombinant DNA according to claim 1 or 2, wherein the said promoter is obtainable from the Delta-0.3TobRB7-5A promoter.

4. A replicon comprising a recombinant DNA according to any one of the claims 1 to 3.

5. The replicon of claim 4, which is a Ti- or Ri-plasmid of an Agrobacterium species.

6. The replicon of claim 4, which is capable of replication in E. coli and Agrobacterium species.

7. An Agrobacterium species comprising a replicon according to any one of claims 5 or 6.

8. A plant genome which comprises a recombinant DNA according to any one of the claims 1 to 3.

9. A plant cell comprising a plant genome according to claim 8.

10. A plant comprising a cell or cells according to claim 9.

11. A plant regenerated from a cell according to claim 9.

12. A plant according to claim 10 or 11, which, as a result of expression of said gene encoding a product that is inhibitory to an endogenous gene, shows reduced susceptibility to a plant parasitic nematode.

13. A plant according to claim 10, which plant belongs to the family Solanaceae.

14. A plant according to claim 13, which plant is Solanum tuberosum.

15. A plant according to any one of the claims 10 to 14, wherein said plant parasitic nematode is a Meloidogyne species.

16. Plant material, such as flowers, fruit, leaves, pollen, seeds, or tubers, obtainable from a plant according to any one of the claims 10 - 15.

17. A method for obtaining a plant with reduced susceptibility to a plant parasitic nematode, comprising the steps of
(1) transforming a recipient plant cell with recombinant DNA according to any one of the claims 1 - 3,
(2) generating a plant from a transformed plant cell,
(3) identifying a transformed plant with reduced susceptibility to said plant parasitic nematode.

18. A method for reducing damage to a crop due to plant parasitic nematodes, by growing plants according to any one of the claims 10-15.

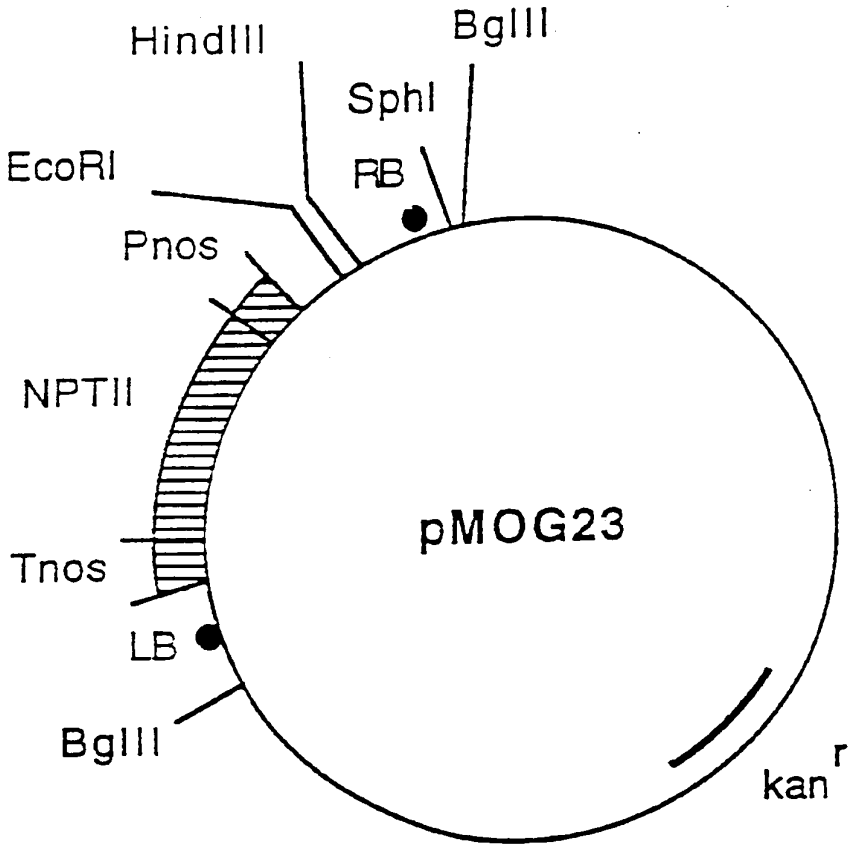
HindIII
BglII
SphI
EcoRI
RB
Pnos
NPTII
pMOG23
Tnos
LB
BglII
kan r

Figure 1

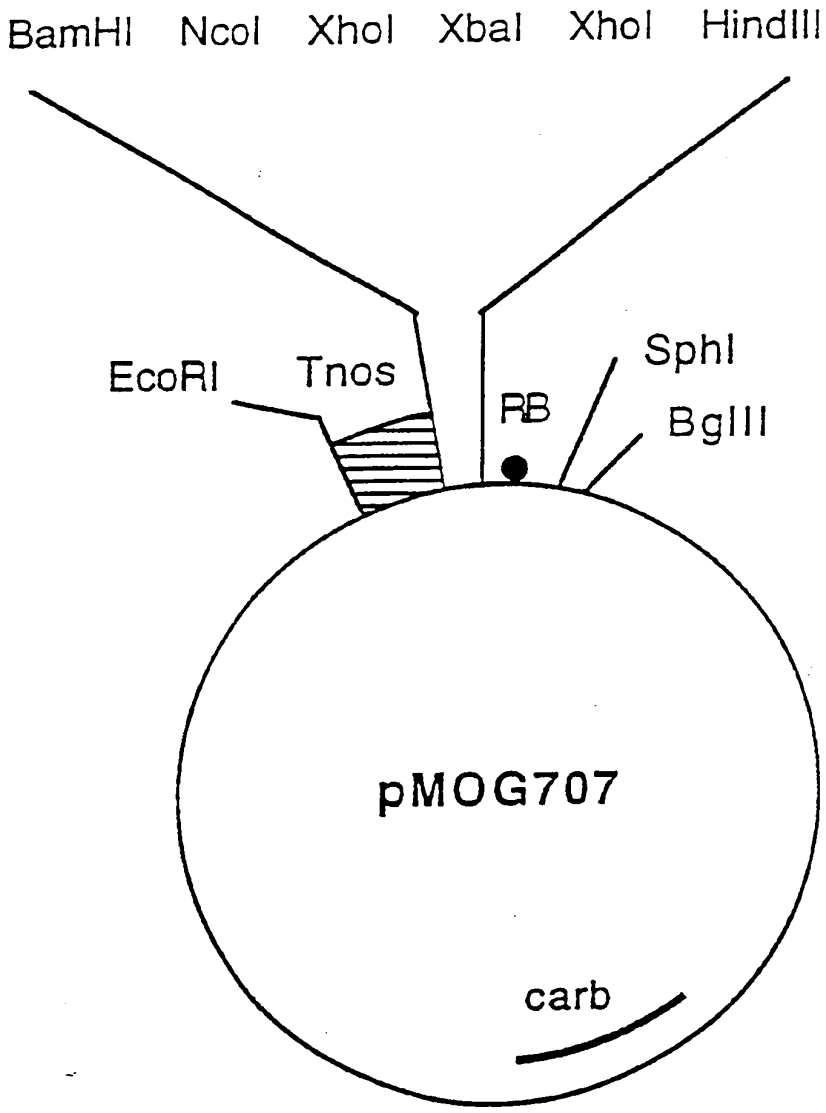
BamHI
NcoI
XhoI
XbaI
XhoI
HindIII
EcoRI
Tnos
RB
SphI
BglII
pMOG707
carb

Figure 2

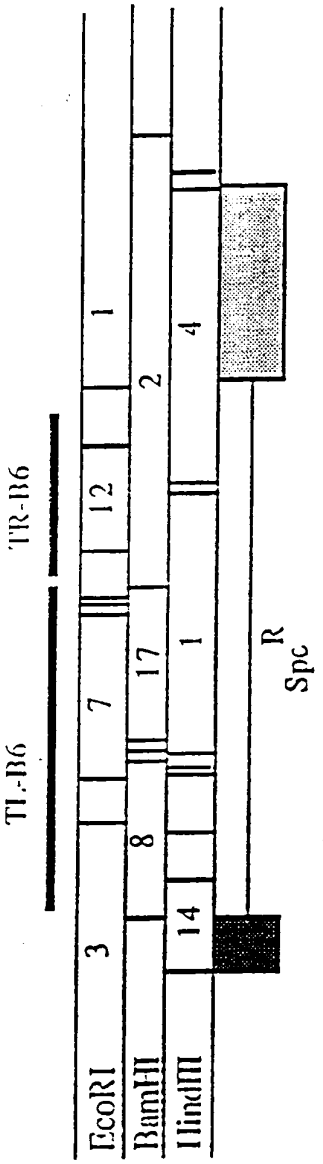

Figure 3

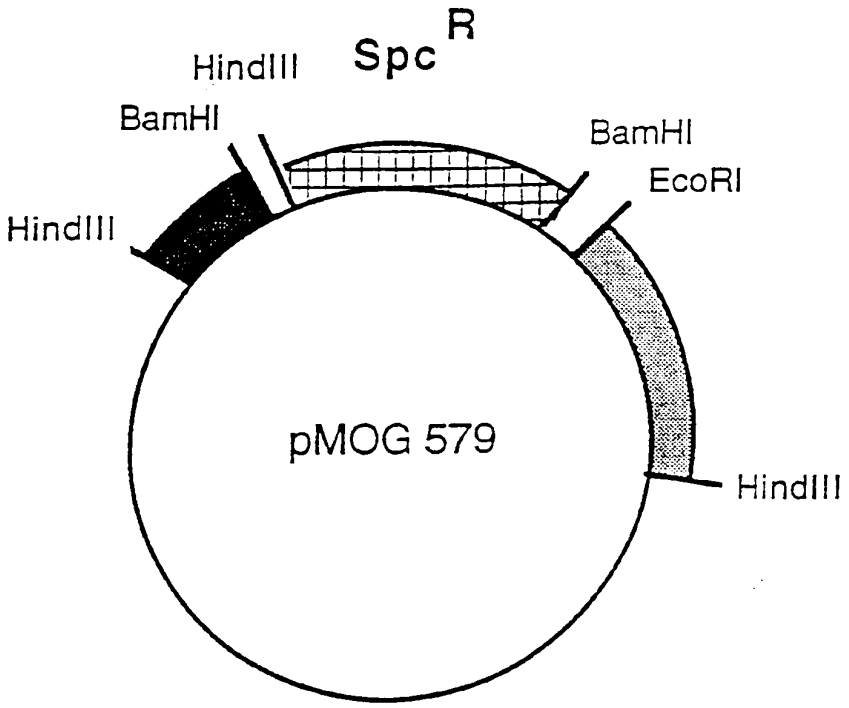

Figure 4

pMOG711 = NADPH-CytP450 reductase ATR1

pMOG712 = NADPH-CytP450 reductase ATR2

pMOG713 = G3P acyltransferase ATS1

pMOG714 = adenine nucleotide translocator

pMOG715 = ß subunit ATP synthase

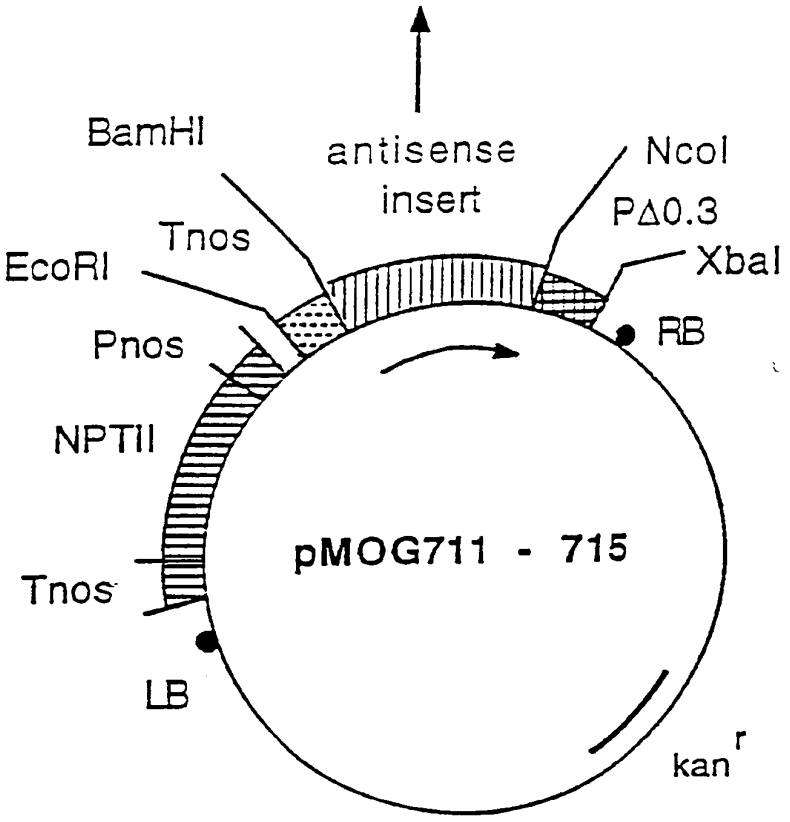

Figure 5

INTERNATIONAL SEARCH REPORT

Inten nal Application No
PCT/EP 93/03091

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/82 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,92 04453 (THE UNIVERSITY OF LEEDS) 19 March 1992 cited in the application see the whole document ---	1,2, 4-14, 16-18
Y	JOURNAL OF CELLULAR BIOCHEMISTRY SUPPLEMENT, vol.0, no.13 D, 1989, NEW YORK page 323 NIEBEL A; INZE D; VAN MELLAERT H; VAN MONTAGU M 'MOLECULAR ANALYSIS OF NEMATODE-INDUCED GIANT CELLS IN POTATO ROOTS' see paragraph M429 --- -/--	1,2, 4-14, 16-18

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

2

Date of the actual completion of the international search	Date of mailing of the international search report
9 February 1994	04 -03- 1994

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Gurdjian, D

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,90 02172 (CALGENE INC.) 8 March 1990 see abstract; claims 1-26	1,2, 4-14, 16-18
A	THE PLANT CELL, vol.3, no.4, April 1991, ROCVILLE US pages 371 - 382 Y.T.YAMAMOTO ET AL. 'Characterization of ...' cited in the application see the whole document	3
A	JOURNAL OF CELLULAR BIOCHEMISTRY SUPPLEMENT, vol.0, no.15 A, 1991, NEW YORK page 56 GURR S J; MCPHERSON M J; ATKINSON H J; BOWLES D J 'IDENTIFICATION OF PLANT GENES EXPRESSED AT THE FEEDING SITE OF THE POTATO CYST NEMATODE' see paragraph A214	1,2, 4-14, 16-18
A	MGG MOLECULAR AND GENERAL GENETICS, vol.226, no.3, May 1991, BERLIN D pages 361 - 366 S.J.GURR ET AL. 'Gene expression in nematode-infected plant roots' cited in the application see the whole document	1,2, 4-14, 16-18
A	EP,A,0 502 730 (NICKERSON BIOCEM LIMITED) 9 September 1992 see claims 1-14	15
A	EP,A,0 480 730 (AMOCO CORP.) 15 April 1992 see abstract; claims 1-26	1
A	EP,A,0 458 367 (CALGENE ,INC) 27 November 1991 see page 4, line 16 - line 37	1,2
P,X	WO,A,92 21757 (PLANT GENETIC SYSTEMS N.V.) 10 December 1992 see the whole document	1-4,6-12

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter...tional Application No
PCT/EP 93/03091

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EP-A-0458367	27-11-91	AU-A-	1301792	03-09-92
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		EP-A-	0240208	07-10-87
		JP-A-	62296880	24-12-87
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WO-A-9221757	10-12-92	NONE		